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2020-10

Moura , C C D M , Fernandes , A M , Aleixo , A , Pereira de Araujo , H F , Mariano , E D F & Wink , M 2020 , ' Evolutionary history of the Pectoral Sparrow Arremon taciturnus : evidence for diversification during the Late Pleistocene ' , Ibis , vol. 162 , no. 4 , pp. 1198-1210 . https://doi.org/10.1111/ibi.128

http://hdl.handle.net/10138/320197
https://doi.org/10.1111/ibi.12813

IBIS
BOU international journal of avian science
*Ibis* (2020), **162**, 1198–1210 doi: 10.1111/ibi.12813

# Evolutionary history of the Pectoral Sparrow *Arremon taciturnus*: evidence for diversification during the Late Pleistocene

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We focus on reconstructing a spatiotemporal scenario of diversification of a widespread South American species, the Pectoral Sparrow *Arremon taciturnus* (Aves: Passerellidae). This species is widely distributed in both the humid and the dry forests of South America and therefore provides an interesting model for understanding the connection between different biomes of South America. We examined nucleotide sequences of the mitochondrial genes Cytochrome b (cyt-b) and NADH subunit 2 (ND2) from 107 specimens, and one nuclear marker (intron 7 of the β-fibrinogen gene) from a subset of samples collected across the distribution ranges of *A. t. taciturnus* and *A. t. nigrirostris*. Six major lineages were recovered in the phylogenies that displayed high levels of variance of allele frequencies and corresponded to distinct geographical locations. The estimation of divergence times provided evidence that diversification of the six lineages of the Pectoral Sparrow occurred throughout the Late Pleistocene across major *cis*-Andean biomes and Amazonian interfluves. Our dataset for *A. taciturnus* provides further evidence that rivers in Amazonia constitute barriers promoting allopatric speciation, with occasional sharing of alleles among lineages, particularly those with adjacent distributions.

**Keywords:** Amazonia, Atlantic Forest, biogeography, forest-associated species, Neotropics.

The South American humid forests (e.g. Atlantic Forest, Amazon Rainforest, Montane Rainforests, Monsoon Forest) are tropical biomes that harbour considerable biodiversity (Ab'Saber 1977). These forest biomes are separated from each other by open biomes including Andean Grassland, Caatinga, Cerrado and Chaco (Werneck 2011). These tracts of open vegetation serve as important barriers influencing the geographical and genetic structure among populations of many species (Batalha-Filho *et al.* 2013). Several studies have highlighted evidence for historical and current connections among the rainforest biomes, for example the presence of relict enclaves of rainforest and gallery forests in the seasonally dry tropical forests in South America (Batalha-Filho *et al.* 2013). Palaeopalynology studies support the evidence of a past connection between the Atlantic Forest and Amazonia (Ledru *et al.* 2005) with subsequent separation during the Tertiary/Lower Pleistocene transition (Werneck 2011). The magnitude of the extent to which past climatic oscillations and subsequent landscape rearrangements have determined the patterns of distribution and driven divergence within species and among members of species-complexes continues to be a focus of investigation to better clarify the evolutionary history of forest-associated species.

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Given the predominant framework that climate and landscape modifications during the Last Glacial Maximum (LGM) underpin geographical structuring within lineages, the Amazonian lowlands are predicted to have shrunk to refugial areas isolated by open biomes that expanded during colder and drier periods (Haffer 1969, Häggi *et al.* 2017, Arruda *et al.* 2018). During these periods, the disjunction of forested areas led to intraspecific differentiation and, over time, to allopatric speciation of isolated populations (Haffer 1969).

Other landscape changes, such as the emergence of river systems in the Neotropics, may have represented barriers to the dispersion of widespread species that inhabited both sides of large rivers (e.g. Amazon and Xingu Rivers), leading to allopatric speciation and thereby resulting in species being endemic to different drainage basins for several taxa. The establishment of the main rivers in the Neotropical region, such as the Amazon and its tributaries, took place between 9 and 2.5 million years ago (Late Miocene to Early Pleistocene; Latrubesse 2012, Hoorn *et al.* 2017) and might have shaped the patterns of ecological and genetic variation observed among birds and other organisms. Indeed, several studies based on reconstructing biogeographical and phylogeographical patterns have highlighted the influence of river systems as barriers leading to vicariance between lineages and, over time, speciation (Aleixo 2004, Burney & Brumfield 2009, Fernandes *et al.* 2012, Ribas *et al.* 2012).

The Pectoral Sparrow *Arremon taciturnus* (Passeriformes: Passerellidae) is a resident species that occurs throughout most of Amazonia and northeastern Brazil, as well as along the base of the Andes in Colombia, Venezuela, Bolivia and Peru (Fig. 1). The species occupies open undergrowth in humid forest or tropical moist lowland forests. *A. taciturnus* was considered to be polytypic, with three currently recognized subspecies: *A. t. axillaris*, occurring along the base of the Andes in Colombia and Venezuela; *A. t. nigrirostris*, recorded in Peru and Bolivia along the eastern slope of the Andes; and *A. t. taciturnus*, extending from southeastern Colombia, east to southern Venezuela and the Guianas, south through Amazonia to central Brazil and northeastern Bolivia, and along the Atlantic coast of Brazil south to Espírito Santo (Jaramillo & de Juana 2017). However, Buainain *et al.* (2017) have shown that *A. t. axillaris* can be phenotypically diagnosed from the remaining subspecies and argued that it should be recognized as a distinct species. In contrast, *A. t. taciturnus* and *A. t. nigrirostris* are polymorphic, with both taxa sharing character states varying from having a complete pectoral band to its absence.

In the present study, we seek to understand the patterns of genetic structure among populations of *A. taciturnus* across the different biomes of the Neotropical region and to infer the influence of barriers to dispersal on the genetic differentiation of *A. t. taciturnus* and *A. t. nigrirostris*. We hypothesize that the major South American rivers that separate the populations of *A. taciturnus* have played a central role as geographical barriers in limiting the extent of gene flow (contact) among lineages, resulting in strong geographical structure across the species range during the Late Pleistocene.

## METHODS

### Sampling

We sampled 107 individuals of *A. taciturnus* from 67 localities (GenBank accession numbers MH218121–MH218364; Fig. 1 and Table S1) comprising both subspecies, and excluded *A. t. axillaris*, which we consider a separate species following Buainain *et al.* (2017). We were not able to sample *A. franciscanus*, the closest extant relative of the *A. taciturnus* complex (Klicka *et al.* 2014). In addition, we included as outgroup taxa, five individuals of *A. flavirostris* and one individual of *A. semitorquatus* (Table S1). Although a complete phylogeny for the genus *Arremon* is lacking, *A. flavirostris* and *A. semitorquatus* have been recovered as phylogenetically close to *A. taciturnus* (Klicka *et al.* 2014). The samples used in this study were either collected in the field or obtained through loans from the following institutions: Universidade Federal da Paraíba, Brazil (UFPB); Paraense Emílio Goeldi Museum, Brazil (MPEG); Louisiana State University Museum of Natural Science, Baton Rouge, LA, USA (LSUMZ); Natural History Museum of Denmark, Denmark (ZMUC); and the Field Museum of Natural History, USA (FMNH). Cytochrome b (cyt-b) and NADH subunit 2 (ND2) sequences from one specimen of *A. taciturnus* available in GenBank (accession numbers KC007559 and KC007573) were also included in the analyses (Table S1).

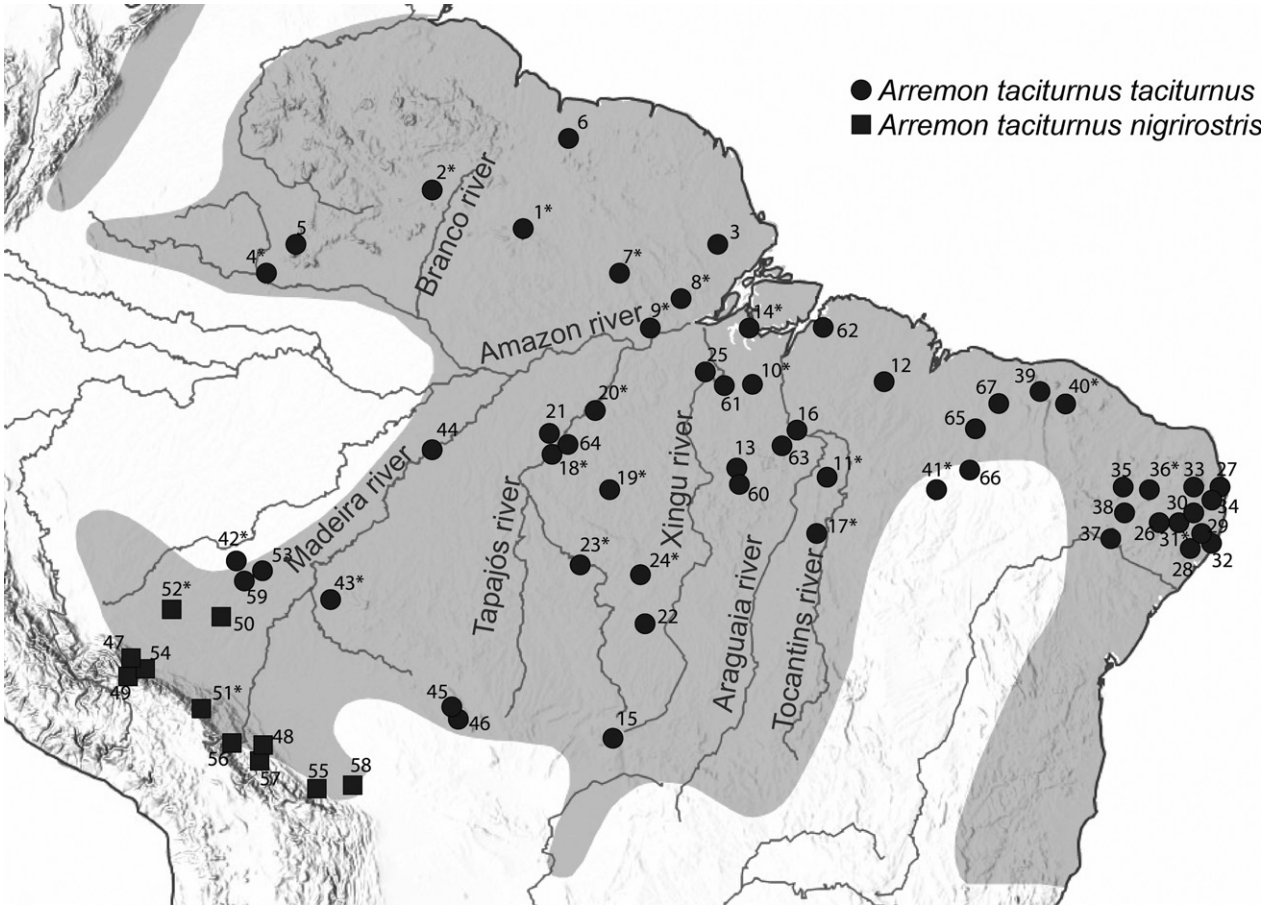


**Figure 1.** Geographical distribution of sampling localities for *Arremon taciturnus* in South America. Circles represent the nominate form *A. t. taciturnus* and squares represent the subspecies *A. t. nigrirostris*; numbers represent the localities detailed in Table S1. Asterisks (*) beside numbers represent the subset of samples amplified for the nuclear gene β-fibint7.

## DNA extraction, PCR-amplification and sequencing

A standard phenol–chloroform method (Green & Sambrook 2012) was used to isolate DNA from muscle. We PCR-amplified two mitochondrial genes, cyt-b and ND2, from 107 individuals, and intron 7 of the nuclear Beta-Fibrinogen gene (β-fibint7) from 35 individuals (GenBank accession numbers of the samples MH218121–MH218364) by polymerase chain reaction (PCR), and sequenced these loci using primers designed by Sorenson *et al.* (1999) and Burney and Brumfield (2009). For some samples, we designed a specific forward primer named Cyf to facilitate the PCR-amplification of cyt-b (Table S2). The cyt-b gene was selected because it has been widely and successfully used in phylogenetic studies of birds and its nucleotide substitution rate has been calibrated (Weir & Schluter 2008). In addition, both ND2 and the nuclear β-fibint7 genes have proven to be suitable for the analysis of avian phylogenetic relationships and elucidation of phylogeographical patterns.

## Phylogenetic analyses and molecular dating

A tree using the alignment of the concatenated mitochondrial and nuclear genes was inferred using the method described by Heled and Drummond (2010) in *BEAST 1.8.0 (Drummond *et al.* 2012). The evolutionary models applied for each gene were defined using JMODELTEST ver. 2.1.7 (Posada 2008); the Akaike information criterion (AIC) supported the model HYK+InvGamma for both mitochondrial genes cyt-b and ND2, and HYK+Gamma for β-fibint7. The divergence time

was fixed according to the cyt-b substitution rate per million years ($2.1 \pm 0.1\%$, 95% confidence interval), and was selected to estimate the substitution rate of the ND2 and the β-fibint7 genes. We used BEAUTI ver. 1.8.0 (Drummond *et al.* 2012) to generate the xml file, and selected a log-normal relaxed clock (Ferreira & Suchard 2008) under a coalescent constant size prior; we ran the analysis for $10^6$ generations and sampled every 1000 trees. Similar parameters were set to run Bayesian inference with the mitochondrial dataset (cyt-b + ND2) and only the ND2 dataset, the latter comprising the larger number of sequences, including those available in NCBI (Table S1). TRACER 1.5 (Rambaut & Drummond 2014) was used to determine the number of generations required to reach stationarity of the posterior distribution. The effective sample size (ESS values) obtained from the Bayesian inference exceeded 200 for all parameters. The posterior distribution of the trees was summarized using TREEANNOTATOR ver. 1.8.0 (Drummond *et al.* 2012) and burnin was set to 80 (8%).

For comparison with the above, we also performed partitioned analyses using MRBAYES ver. 3.2.6 (Ronquist *et al.* 2012) for the mitochondrial genes. To select the best-fit partitioning scheme for each gene we used the software PARTITIONFINDER ver. 2.1.1 (Lanfear *et al.* 2012), with the following models recovered for each partition: cyt-b 1st codon (HKY+I+G); cyt-b 2nd codon and ND2 2nd codon (TrN+I); cyt-b 3rd codon (TrN+G); ND2 1st codon (HKY+I); and ND2 3rd codon (GTR+G). Two independent runs each with four chains and set to $10^7$ generations were run with trees sampled every 1000 generations and a burnin of 25%. The analysis was carried out using the CIPRES platform (Miller *et al.* 2010).

A maximum likelihood (ML) analysis was performed in MEGA 6.0 (Tamura *et al.* 2013) with 1000 bootstrap pseudoreplications, under the HYK+InvGamma model of nucleotide substitution with nearest-neighbour-interchange and strong branch swapping.

### Population genetics and historical demography

We performed all demographic tests based on the mitochondrial dataset for the six main lineages obtained in the phylogenetic analyses (see Results). DNASP ver. 5.0 (Librado & Rozas 2009) was used to estimate the following population parameters: nucleotide diversity ($\pi$), haplotype diversity ($h$), Tajima's $D$ and Fu's $F_S$ statistics. To further evaluate population differentiation, we estimated pairwise $F_{ST}$ using ARLEQUIN ver. 3.5.2.2 (Excoffier & Lischer 2010).

Mean pairwise uncorrected-p distances were calculated among lineages using MEGA ver. 6.0 (Tamura *et al.* 2013). To visualize genealogical relationships among the populations, a haplotype network was reconstructed with maximum parsimony using the median-joining algorithm in the program NETWORK ver. 5 (Bandelt *et al.* 1999) based on the mitochondrial dataset. Using DNAsp we determined the degree of genetic differentiation between populations of *A. taciturnus* located across several rivers (Amazon, Branco, Tocantins, Araguaia, Xingu, Tapajós and Madeira; Fig. 1) using the fixation index ($F_{ST}$), the average number of nucleotide substitutions per site between populations (*Dxy*), the number of nucleotide substitutions per site between populations (*Da*), the global genetic differentiation between populations ($N_{ST}$) and the pairwise p-distance.

## RESULTS

### Phylogenetic analyses

Six major lineages were consistently recovered in *A. taciturnus* by phylogenetic inference using the mitochondrial dataset (Figs 2, Figs S1, S2), via the haplotype network (Fig. 3), and in the multilocus tree (Fig. 4). Clades are associated with different bioregions as follows: birds in lineage I are mostly confined to region A (northern Amazon River); lineage II to region B (Cerrado); lineage III to region C (most of the Tapajós–Xingu Interfluve); lineage IV to region D (Atlantic Forest); lineage V to region E (Caatinga); and lineage VI to region F (southwest Amazonia) (Fig. 2). The median-joining network using the concatenated mitochondrial genes recovered concordant results (Fig. 3).

Only lineage IV was recovered as monophyletic with our sampling, although with little support (Figs 2, 4 and S1, S2). Despite the overall lack of resolution and support of the basal nodes uncovered in the phylogenetic analyses, two main groups were evident: one grouping lineages I and II, and the second grouping lineages III, IV, V and VI (Fig. 4). The haplotype network (Fig. 3) reflects

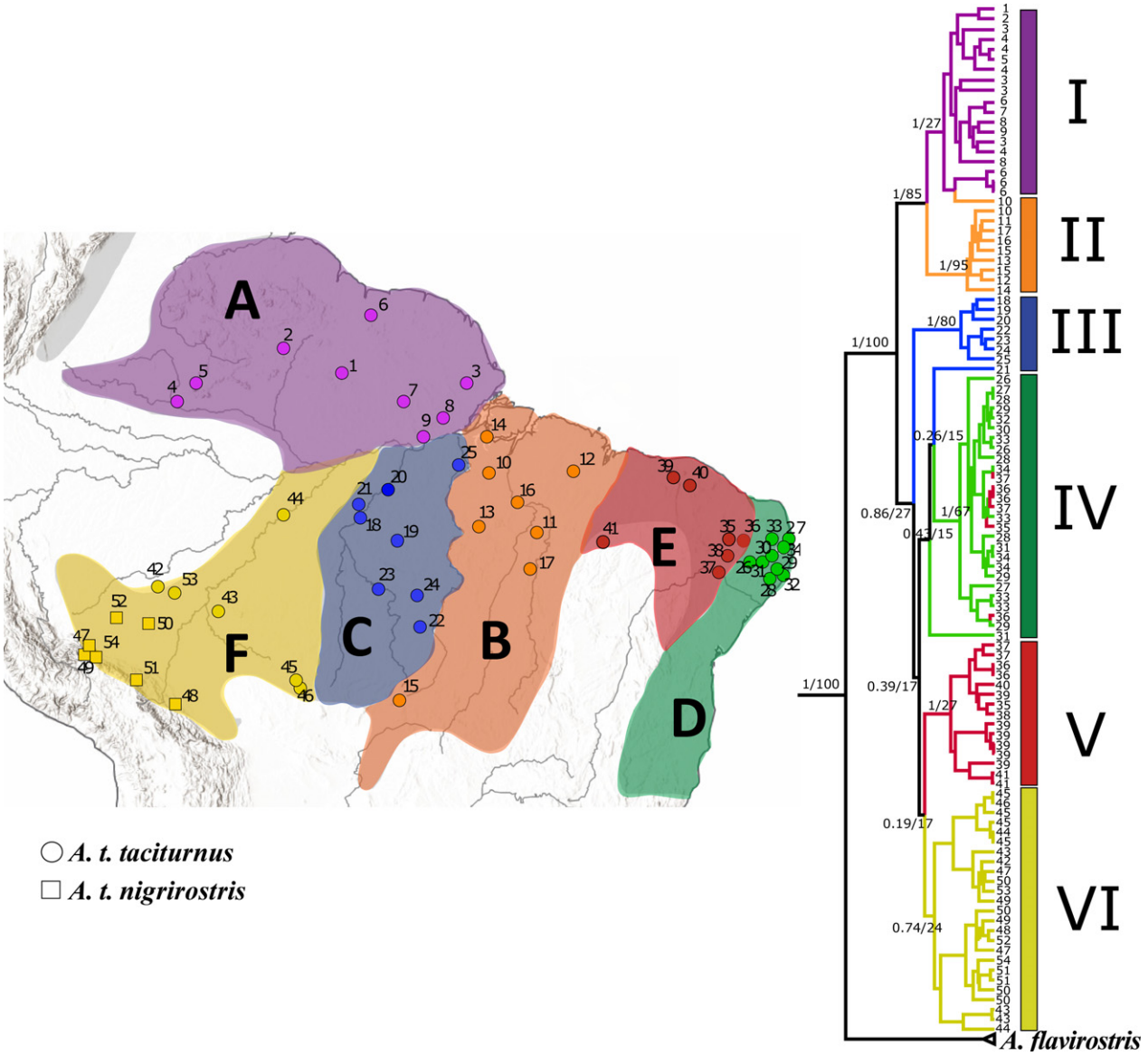


**Figure 2.** Phylogenetic relationships of *Arremon taciturnus* inferred using the two mitochondrial genes, cyt-b and ND2 (in total 2048 bp). The topology displayed corresponds to the tree obtained using Bayesian inference; posterior probabilities and ML bootstrap support values are given at the nodes. Left, map with the geographical distribution of the sampling sites. Colours on the map correspond to respective clades in the phylogeny (right), while numbers correspond to sampling sites (Table S1). Lineages are represented by I–VI and regions by A–F.

the population structure across major *cis*-Andean biomes and Amazonian interfluves, albeit with some degree of apparent gene flow among groups, particularly those with adjacent distributions. In contrast to the phylogenetic analyses based on the mitochondrial dataset, a lack of population structure was observed in the nuclear β-fibint7 phylogenetic tree (Fig. S3) and haplotype network (Fig. S4).

## Molecular dating

According to the multilocus tree, the split between *A. taciturnus* and *A. flavirostris* is estimated to have taken place *c.* 3.2 million years ago (Mya) during the Pliocene (Fig. 4). The multilocus tree recovered six main lineages that diverged throughout the Late Pleistocene period (0.57–0.35 Mya). The first split within the

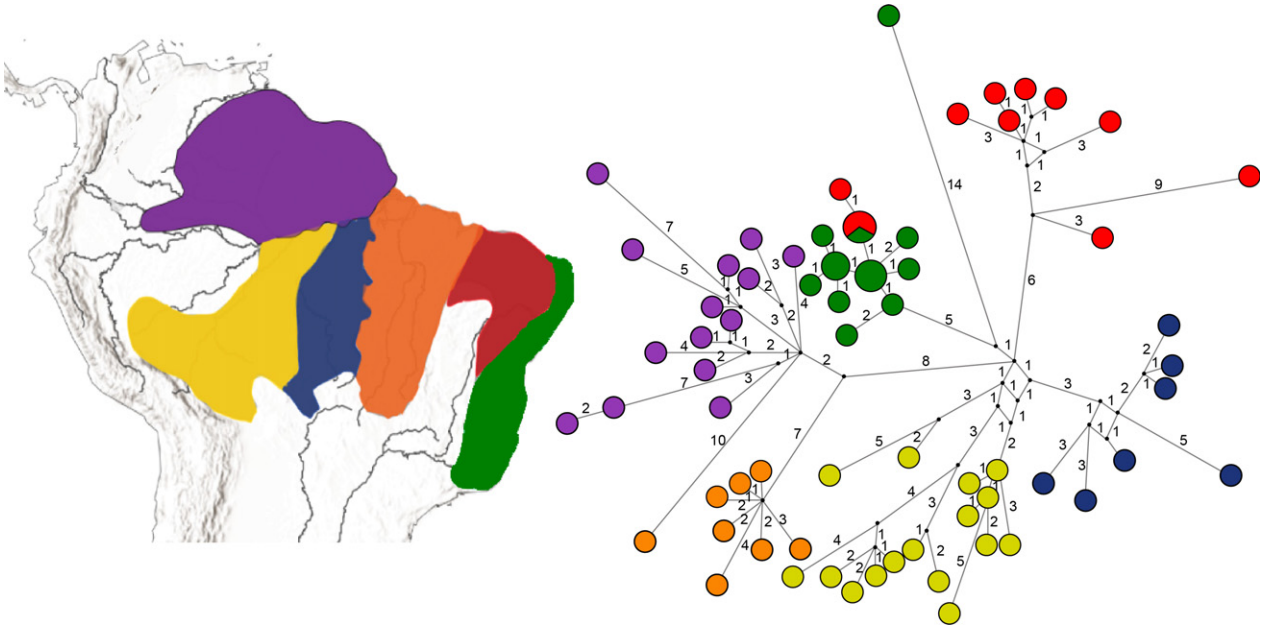

**Figure 3.** Median joining haplotype network of *Arremon taciturnus* based on mitochondrial DNA (cyt-b and ND2). Colours correspond to each lineage with the distribution range represented by the map on the left, circles correspond to sample size, and the numbers represent the number of nucleotide substitutions separating lineages.

lineages of *A. taciturnus* took place *c.* 0.57 Mya and separated two main groups: one including lineages I and II distributed across the northern Amazon River and Cerrado bioregion, and a second group comprising the remaining lineages encompassing the Tapajós–Xingu Interfluve, Atlantic Forest, Caatinga and southwestern Amazonia. Lineages I and II split around 0.39 Mya, whereas the clade consisting of lineages III–VI included several cladogenetic events dated to 0.45 Mya.

## Sequence polymorphism and historical demography

The combined mitochondrial dataset (2048 bp) recovered 67 haplotypes with 160 polymorphic sites, 84 of which were parsimony-informative. Six major lineages were consistently recovered in the phylogenetic analysis of *A. taciturnus*.

The haplotypic diversity for this dataset was high ($h = 0.989 \pm 0.005$) and the nucleotide diversity low ($\pi = 0.012 \pm 0.0002$); the same pattern can be observed within each clade (Table 1). We also sequenced 562 bp of the nuclear intron β-fibint7 from 35 individuals belonging to the major lineages, from which we recovered five haplotypes, differentiated by five variable sites, and two parsimony-informative sites. Allelic diversity was high ($h = 0.585 \pm 0.045$) and nucleotide diversity low ($\pi = 0.0018 \pm 0.0002$).

Although the neutrality tests were non-significant in some of the lineages, significantly negative values of Fu's and Tajima's *D* tests are consistent with recent demographic expansion for the mitochondrial dataset (Table 1). Pairwise fixation indexes ($F_{ST}$) revealed moderate to high differentiation, with $F_{ST} > 0.10$ and significant values ($P < 0.05$) for all lineage comparisons, highlighting the differentiation between lineages, and supporting the deep genetic structure among regional clades (Table 2). The average pairwise uncorrected-p distances between lineages ranged from 0.8 to 1.5% (Table 3), with 1% as the mean pairwise difference.

Our results of genetic differentiation for *A. taciturnus* across riverine barriers support a reduction of gene flow between populations situated on either side of the following rivers: Amazon ($F_{ST} = 0.4707$), Tocantins ($F_{ST} = 0.5728$), Xingu ($F_{ST} = 0.6385$), Tapajós ($F_{ST} = 0.3645$), Madeira ($F_{ST} = 0.2681$) and Branco ($F_{ST} = 0.1529$), with pairwise parsimony distances varying from 0.6 to 1.7%, whereas the Araguaia River ($F_{ST} = 0.0000$)

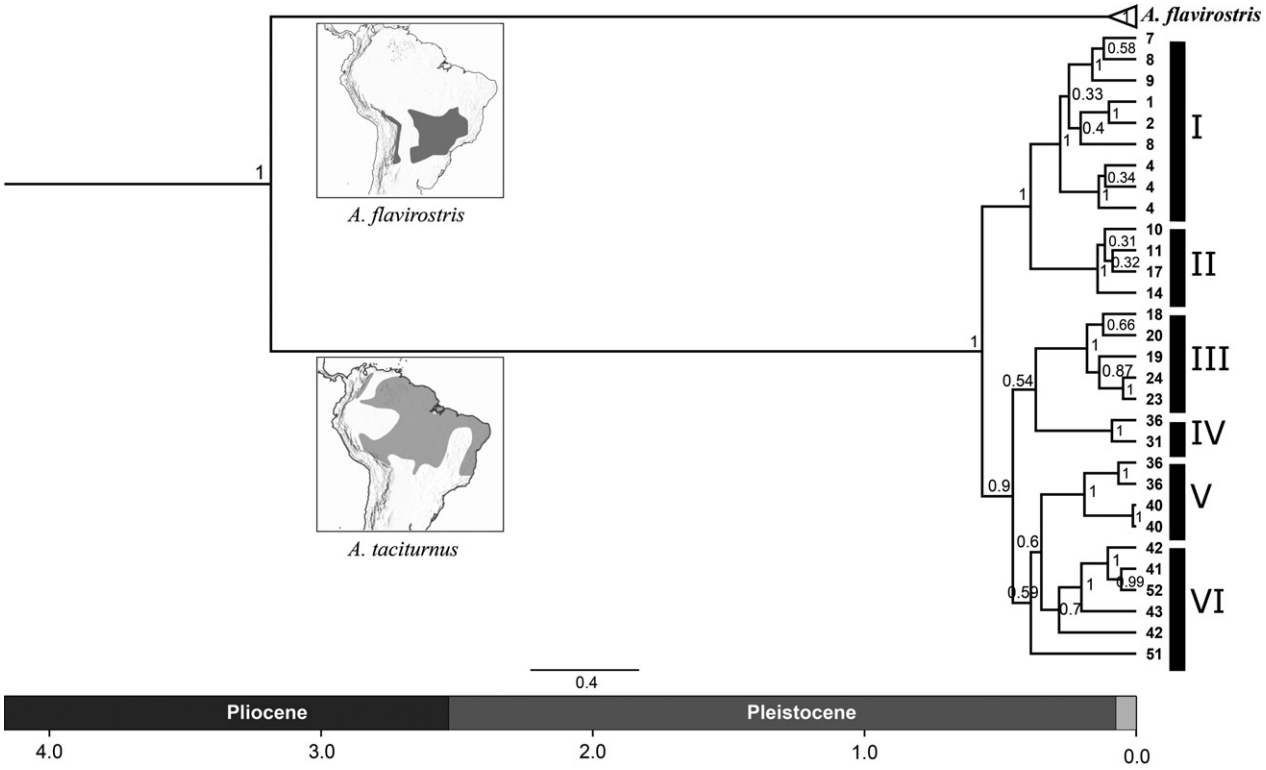


**Figure 4.** Multilocus tree based on the mitochondrial and nuclear genes of *Arremon taciturnus*, using as outgroup the species *A. flavirostris* (scale axis estimated at million years ago (Mya)) calculated using BEAST ver. 1.8.0. The numbers at the tip labels correspond to the localities plotted on the map (Fig. 1 and Table S1). Lineages are represented by I–VI and correspond to the following regions: Lineage I – Northern Amazon River; Lineage II – Belém + Xingu + Cerrado; Lineage III – Tapajós–Xingu Interfluve; Lineage IV – Atlantic Forest; Lineage V – Caatinga; Lineage VI – Southwest Amazonia (Rondônia + Inambari). Posterior probabilities are given at the nodes. The maps show the range of distribution from the species *A. taciturnus* and *A. flavirostris* (BirdLife International and Handbook of the Birds of the World 2019).

**Table 1.** Genetic diversity and demographic population parameters for the lineages recovered through the phylogenetic reconstruction for the species *Arremon taciturnus* using nucleotide sequences of the mitochondrial Cytochrome b and ND2 genes (2048 bp).

| Lineage | *n* | No. of haplotypes | *h* | π | Tajima's *D* | Fu's F ($F_S$) |
|---|---|---|---|---|---|---|
| All populations | 107 | 83 | 0.989 ± 0.005 | 0.012 ± 0.0002 | −1.850 $P < 0.05$ | −66.368 $P < 0.05$ |
| Lineage I | 20 | 17 | 0.988 ± 0.021 | 0.005 ± 0.0004 | −1.669 $P > 0.05$ | −6.876 $P < 0.05$ |
| Lineage II | 10 | 10 | 1.000 ± 0.045 | 0.004 ± 0.0010 | −2.087 $P < 0.01$ | −5.065 $P < 0.05$ |
| Lineage III | 8 | 8 | 1.000 ± 0.039 | 0.005 ± 0.0009 | −1.153 $P > 0.10$ | −2.317 $P > 0.05$ |
| Lineage IV | 35 | 23 | 0.956 ± 0.021 | 0.008 ± 0.0021 | −1.551 $P > 0.10$ | −3.492 $P < 0.05$ |
| Lineage V | 9 | 7 | 0.944 ± 0.070 | 0.006 ± 0.0007 | 0.314 $P > 0.10$ | 0.063 $P > 0.05$ |
| Lineage VI | 25 | 21 | 0.983 ± 0.017 | 0.005 ± 0.0003 | −0.964 $P > 0.10$ | −8.565 $P > 0.05$ |

*D*, Tajima's *D*; Fu's $F_S$; *h*, haplotype diversity; *n*, number of samples; π, nucleotide diversity.

did not represent a barrier to gene flow for *A. taciturnus*, with a pairwise distance of 0.4% (Table 4).

## DISCUSSION

*Arremon taciturnus* is a forest-associated species that occupies both the humid and the dry forests of South America, an ideal exemplar species with which to infer historical processes related to the interplay between these adjacent habitats in *cis*-Andean South America. To date, phylogeographical studies of Neotropical birds have tended to focus on lineages endemic to either humid forest (Amazon Rainforest and Atlantic

**Table 2.** Genetic distance calculated using pairwise $F_{ST}$ values for the six major lineages recovered through the phylogenetic analysis of the *Arremon taciturnus* dataset using nucleotide sequences of the Cytochrome b and ND2 genes (all pairwise $F_{ST}$ values presented are significant, $P < 0.05$).

| | Lineage I | Lineage II | Lineage III | Lineage IV | Lineage V | Lineage VI |
|---|---|---|---|---|---|---|
| Lineage I | - | | | | | |
| Lineage II | 0.36339 | - | | | | |
| Lineage III | 0.41927 | 0.58713 | - | | | |
| Lineage IV | 0.33451 | 0.45542 | 0.27143 | - | | |
| Lineage V | 0.50451 | 0.60424 | 0.47412 | 0.33724 | - | |
| Lineage VI | 0.37265 | 0.53380 | 0.29313 | 0.24399 | 0.34048 | - |

Lineage I: Northern Amazon River; Lineage II: Belém + Xingu + Cerrado; Lineage III: Tapajós–Xingu Interfluve; Lineage IV: Atlantic Forest; Lineage V: Caatinga; Lineage VI: Southwest Amazonia (Rondônia + Inambari).

**Table 3.** Pairwise uncorrected parsimony distances between and within lineages recovered through the phylogenetic analysis of the species *Arremon taciturnus* using nucleotide sequences of the mitochondrial gene Cytochrome b.

| | Lineage I | Lineage II | Lineage III | Lineage IV | Lineage V | Lineage VI |
|---|---|---|---|---|---|---|
| Lineage I | 0.006 | | | | | |
| Lineage II | 0.011 | 0.005 | | | | |
| Lineage III | 0.011 | 0.014 | 0.005 | | | |
| Lineage IV | 0.012 | 0.015 | 0.010 | 0.004 | | |
| Lineage V | 0.010 | 0.013 | 0.009 | 0.008 | 0.006 | |
| Lineage VI | 0.011 | 0.013 | 0.009 | 0.010 | 0.008 | 0.007 |

Lineage I: Northern Amazon River; Lineage II: Belém + Xingu + Cerrado; Lineage III: Tapajós–Xingu Interfluve; Lineage IV: Atlantic Forest; Lineage V: Caatinga; Lineage VI: Southwest Amazonia (Rondônia + Inambari).

Forest; Cabanne *et al.* 2013, Fernandes *et al.* 2014, Thom & Aleixo 2015) or dry forests (Costa 2003, Werneck *et al.* 2012), precluding more detailed insights into correlated historical shifts associated with both of these habitat types. Our results uncovered significant phylogeographical structure in *A. taciturnus*, supporting geographically structured mitochondrial DNA (mtDNA) phylogenies without nuclear gene corroboration. Sharing of alleles was observed between major geographical lineages, particularly those with adjacent distributions. This pattern has been observed for populations that have recent histories of isolation (Zink & Barrowclough 2008). The longer coalescence times of nuclear genes (*c.* 4 times longer than mtDNA) (Zink & Barrowclough 2008) result in lagging indicators of population structure changes, rather than refuting the mtDNA evolutionary divergence. Female dispersal distances do surpass those of males, reducing this effect (Zink & Barrowclough 2008). However, there is no evidence of female phylopatry recorded in the species studied.

## Systematic implications

The sampling of our study does not allow for a complete overview of the systematics of the *A. taciturnus* species complex, which also includes two taxa not sequenced in the present study (*A. franciscanus* and *A. t. axillaris*). Buainain *et al.* (2017) showed that *A. t. axillaris* is consistently distinct morphologically and vocally from the remaining populations attributed to *A. taciturnus*, and it could be regarded as a separate species. The third taxon of the *A. taciturnus* complex included in our sampling, *A. semitorquatus*, clustered consistently as the sister taxon to all samples of *A. taciturnus* sequenced by us, although with low statistical support, which could be due to the sampling of only one individual of *A. semitorquatus* (sequenced for ND2 only; Fig. S2). The same topology showing a sister relationship between *A. taciturnus* and *A. semitorquatus* was supported by an extensive mitochondrial dataset of Passerellidae (Klicka *et al.* 2014), but not by another study focused on *Arremon flavirostris* which sampled all members of the *A. taciturnus* complex, except

| Western × Eastern riverbanks | Pairwise genetic distance | $F_{ST}$ | *Dxy* | *Da* | $N_{ST}$ |
|---|---|---|---|---|---|
| Amazon River | 0.012 | 0.4707 | 0.011 | 0.005 | 0.4724 |
| Branco River | 0.006 | 0.1529 | 0.006 | 0.0009 | 0.1530 |
| Tocantins River | 0.017 | 0.5728 | 0.016 | 0.0092 | 0.5749 |
| Araguaia River | 0.004 | 0.0000 | 0.004 | 0.0000 | –0.0003 |
| Xingu River | 0.015 | 0.6385 | 0.014 | 0.0092 | 0.6406 |
| Tapajós River | 0.011 | 0.3645 | 0.010 | 0.0037 | 0.3652 |
| Madeira River | 0.006 | 0.2681 | 0.006 | 0.0017 | 0.2683 |

*Da*, number of nucleotide substitutions per site between populations; *Dxy*, average number of nucleotide substitutions per site between populations; $F_{ST}$, fixation index, a measure of population differentiation; $N_{ST}$, global genetic differentiation among populations.

**Table 4.** Genetic differentiation between populations of *Arremon taciturnus* separated by riverine barriers (comparisons between populations located on either side of the following rivers: Amazon, Branco, Tocantins, Araguaia, Xingu, Tapajós and Madeira), based on nucleotide sequences of the mitochondrial genes Cytochrome b and ND2.

*A. t. axillaris* (Trujillo-Arias *et al.* 2017). Therefore, basal phylogenetic relationships within the genus *Arremon* are still poorly understood, and the same applies to the *A. taciturnus* complex, whose monophyly has yet to be corroborated. Our molecular results support that the subspecies *A. t. taciturnus* and *A. t. nigrirostris* do not represent distinct lineages, mirroring the results obtained based on both plumage and vocal characters, whereby these taxa could not be reciprocally diagnosed. Neither the morphological nor the vocal similarities documented between the populations of *A. taciturnus* by Buainain *et al.* (2017) were consistent with the phylogenetic relationships recovered among the six genetic lineages by our data, supporting the conclusion that *A. t. nigrirostris* should be synonymized with *A. t. taciturnus*.

## Historical biogeography

The estimated time tree favours a scenario of intense diversification via vicariance in *A. taciturnus* across major *cis*-Andean South American habitats and Amazonian interfluves over the last 0.5 Ma. Interestingly, despite the existence of a phylogeographical structure, most geographical lineages were not reciprocally monophyletic, indicating a lack of complete lineage sorting probably due to recent differentiation. This phylogeographical pattern is intermediate between that detected for typical understorey upland *terra firma* species, which almost invariably show high degrees of phylogeographical structure (Capurucho *et al.* 2013, Thom & Aleixo 2015, Araújo-Silva *et al.* 2017), and that uncovered for seasonally flooded forest taxa, which exhibit little or no phylogeographical structure across large areas (Aleixo 2006, Cadena *et al.* 2011).

The six major geographical lineages identified in the estimated phylogenies and the parsimony network for *A. taciturnus* provide evidence for significant genetic structure across major morphoclimatic domains and interfluves in northern South America. The species tree topology for *A. taciturnus* comprises analogous area relationships to other phylogenetic studies (Aleixo & Rossetti 2007, Martins *et al.* 2009, Milá *et al.* 2009, Harvey & Brumfield 2015, Rocha *et al.* 2015, Araújo-Silva *et al.* 2017). The incomplete sorting of alleles observed in the dataset is associated with a possible secondary contact between lineages of the Atlantic Forest, Caatinga and the central Tapajós–Xingu Interfluve (Fig. 2). According to the phylogenies recovered, some birds in lineages I (area A – north of the Amazon River) and IV (area D – Atlantic Forest) were collected from areas where other haplotypes dominated, pointing to the sharing of alleles between distinct lineages.

That *A. taciturnus* is a typical humid forest edge and dry forest species with a wide distribution in both humid forest (Amazon Rainforest and Atlantic Forest) and more open habitats (Caatinga and Cerrado) (Jaramillo & de Juana 2017) may account for this distinct phylogeographical pattern. Similar patterns of divergence with incomplete sorting of alleles have been detected in other Neotropical species (Milá *et al.* 2009, Gutiérrez-Pinto *et al.* 2012, Harvey & Brumfield 2015) and highlight the relevance of adaptive processes and the effect of ecological transition zones in structuring bird communities (Price 1991, Milá *et al.*

2009). We suppose that relevant processes in the diversification history of the Neotropical species, such as hybridization, introgression and gene flow, will be more evident with the use of genomic datasets.

Although *A. taciturnus* is a non-migratory bird, this species inhabits both dry and humid forests, including sites up to 1000 m in elevation. Consequently, whereas some well-differentiated lineages (II, IV and V) are not currently separated by any major geographical barriers, others (I, II, III and VI) replace each other across major Amazonian rivers (Figs 1 and 2). The phylogenetic relationship suggests that the population structure observed in our mitochondrial dataset might be the result of isolation-by-distance, as *A. taciturnus* displays territorial behaviour and the rivers act as secondary barriers to contact between populations of *A. taciturnus*, which is easily reconciled with the observed pattern of differentiation across rivers, particularly as recent studies have suggested the late Pleistocene as an intense period of drainage reorganization in western and central Amazonia (Hayakawa & Brumfield 2015, Rossetti *et al.* 2015). Despite the 'river effect', even major physical barriers, such as the lowermost Amazon River, have not prevented sharing of alleles between *A. taciturnus* populations, as demonstrated by the lack of reciprocal monophyly between lineages I and II. The same can be observed around the headwaters of the Tapajós River, where lineages II, III and VI replace each other within a comparatively small geographical area (Fig. 2).

The molecular divergence date estimated between lineages I and II (0.39 Ma, confidence interval of 0.27–0.52 Ma), located on opposite banks of the Amazon River, suggests that divergence took place after the establishment of the river's main course, which is estimated to have occurred by the Plio-Pleistocene at the latest (Campbell *et al.* 2006, Hoorn *et al.* 2017). Therefore, the genetic data support a scenario of recent dispersal across the lowermost Amazon, possibly due to Quaternary climatic changes influencing sea level and consequently the discharge and speed of flow of Amazonian rivers (Latrubesse 2012). These events played an important role in the formation of marine terrace deposits, potentially allowing passage in the north–south direction across the lowermost Amazon River, which could have been facilitated by the Marajó Archipelago. The lack of reciprocal monophyly between lineages I and II suggests that the time of the crossing is so recent that both lineages have not yet fully coalesced for the molecular markers sequenced. Lineage I is the only group situated to the north of the Amazon River, and hence a south–north crossing would be a more parsimonious alternative.

However, explaining high genetic divergence between current parapatric lineages replacing each other in easternmost Amazonia and the Cerrado, Caatinga and Atlantic Forest is challenging, given the absence of modern physical barriers separating the populations located in these bioregions. Therefore, past ecological barriers must have played a role in accounting for the observed phylogeographical structure in *A. taciturnus*.

All events of diversification within this species were estimated to have taken place in the Late Pleistocene period, including the isolation of Atlantic Forest lineages, which might have been the result of the glacial periods (Costa 2003, Rull 2011). The Pleistocene climatic oscillation can be associated with habitat fragmentation and establishment of biomes in the Neotropical area, consequently influencing population structure in *A. taciturnus*. Our data do not allow us to determine whether this purported expansion occurred from west to east or in the opposite direction, but these alternative scenarios could be tested with more powerful genomic datasets.

Our results support the idea that despite their genetic differentiation, Caatinga and Atlantic Forest lineages share mitochondrial haplotypes, indicating recent contact between them. This is reinforced by the existence of forest enclaves derived from recurrent Atlantic Forest expansions during Pleistocene humid cycles, and Caatinga/dry forests retractions during cold periods (Prado & Gibbs 1993). In addition, Ab'Saber (1977) have proposed that a few enclaves of forest could have withstood the expansion of dry forests during Pleistocene arid cycles, whereas others would probably have shrunk due to the expansion of semi-arid climate and replacement by Caatinga vegetation. Therefore, the complex relationships between these biomes created conditions for several episodes of contact and gene flow between Atlantic Forest and Caatinga, as supported by our results.

Overall, the spatiotemporal evolutionary pattern estimated here for *A. taciturnus* shows that lineages from dry and gallery forests (i.e. geographical

lineages II and V) are not each other's closest relatives, and that each is more closely related to humid Amazonian and Atlantic Forest lineages (Fig. 2). Along with other evidence discussed above, this demonstrates a tight historical connection between these distinct, albeit adjacent forest types, in addition to supporting a major disconnection between different sectors of Amazonian humid forests by drier vegetation types (Cardoso da Silva 1995). The major split detected in *A. taciturnus*, separating lineages I–II from lineages III–VI is dated to 0.57 Ma (confidence interval of 0.43–0.73 Ma) and could be explained by the 'central dry corridor', which supposedly bisected Amazonian humid forests into an eastern and a western block (Cardoso da Silva 1995). The appearance of this corridor could have allowed for the expansion of the Cerrado lineage (II) into easternmost Amazonia (the Xingu and Belém areas of endemism) and a later crossing of the Amazon River into the Guiana shield (with the differentiation of lineage I), as discussed above. At the same time, the corridor could have separated lineages III–VI into two major groups, one in central and western Amazonia (lineages III and VI), and another in the Caatinga and the Atlantic Forest. Unfortunately, an overall lack of support and resolution characterize the estimated basal relationships among lineages III–IV, making it impossible to evaluate this sequence of splitting events properly. Again, future studies with better sampling in terms of markers could test this hypothesis.

C.C.M.M., A.A. and A.M.F. were supported by a PhD scholarship from CAPES/*Ciências sem fronteiras* (Grant number 1104-13/06), a research productivity fellowship from CNPq (Grant number #306843/2016-1) and a research grant support from FACEPE (Grant number APQ-0641-2.04/18), respectively. We thank the curators of the following collections for permitting us to use tissues under their care in this phylogeographical study: FMNH, Chicago, USA; LSUMZ, Baton Rouge, USA; MPEG, Belém, Brazil; and ZMUC, Copenhagen, Denmark. The research was conducted in the Institute of Pharmacy and Molecular Biotechnology, Heidelberg University. We also thank Hedwig Sauer-Gürth for laboratory support and the preparation of solutions, and Tibério Burlamaqui for supporting us with essential advice on some of the bioinformatics analysis. The authors confirm that there are no known conflicts of interest associated with this publication. We are grateful to the two anonymous reviewers and the editor Rauri Bowie who contributed greatly to the improvement of the manuscript.

## DATA AVAILABILITY STATEMENT

The sequences were uploaded to NCBI and they will be released as soon as the manuscript is accepted.

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Received 19 April 2018;
revision accepted 2 January 2020.
Associate Editor: Sandi Willows-Munro.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Sampling locality, voucher number, respective clades and institutions of origin of samples of *Arremon taciturnus* and outgroups (*A. flavirostris* and *A. semitorquatus*) used in this study. GenBank accession numbers MH218121–MH218364.

**Table S2.** List of primers used to amplify the mitochondrial genes cyt-b and ND2, and the nuclear gene β-fibint7 of the samples from *Arremon taciturnus*.

**Figure S1**. Bayesian inference based on the mitochondrial dataset (cyt-b and ND2) of *Arremon taciturnus* using the following partition scheme: Cyt-b 1st codon (HKY+I+G); Cyt-b 2nd codon and ND2 2nd codon (TrN+I); Cyt-b 3rd codon (TrN+G); ND2 1st codon (HKY+I); and ND2 3rd codon (GTR+G); including as outgroup *A. flavirostris*.

**Figure S2.** Bayesian inference tree based on the ND2 dataset of *Arremon taciturnus*, including as outgroup *A. flavirostris* and *A. semitorquatus*.

**Figure S3.** Bayesian inference based on the nuclear gene β-fibint7 of *Arremon taciturnus*, including as outgroup *A. flavirostris*.

**Figure S4.** Median joining haplotype network of *A. taciturnus* based on the nuclear gene β-fibint7 locus.